AN INVESTIGATION OF NATIVE FLORIDA MALE SALIENTIA AS TEST ANIMALS FOR EARLY PREGNANCY DIAGNOSIS

Keith L. Hansen
Stetson University

A Boston physician, Stanley L. Robbins (1948) began a paper dealing with the various pregnancy tests thus, "It is remarkable that a condition so essentially benign and self-limited as pregnancy, which must inevitably become obvious in the due course of time, has evoked so much interest in its early diagnosis." Today the existing tests for pregnancy may be classified into three main categories: biological assays, chemical assays, and skin tests. Of these three, the biological tests are the only method which has proved accurate and reliable. The biological assays for the determination of early pregnancy are based on the fact that pregnancy urine contains hormones which stimulate the gonads of certain of the lower animals.

The earliest test of this nature was the Aschheim-Zondek (1928) test of 1928. This test employed the female mouse and later the female rat as test animals. In 1931 a modification of this first test was made by Friedman (1931), using the female rabbit. The amphibian was utilized for the first time by Shapiro and Zwarenstein (1934) in 1933. These investigators found that the female South African clawed frog, *Xenopus laevis,* could be used in early pregnancy determination.

In March, 1947 Carlos Galli-Mainini (1947) first showed that the male South American toad, *Bufo arenarum* Hensel, would react to the gonadotropic hormone of pregnancy urine by the release of sperm. Since that date males of the following species of salientia have been used for this test: *Xenopus laevis* used unsuccessfully (Robbins, S. L., and Parker, F., 1948); *Rana pipiens* (Wiltberger, P. B., and Miller, D. F., 1948); *Bufo paracnemis, Bufo marinus, Bufo crucifer, Calyptocephala gayi, Leptodactylus ocellatus, Odontophrynus* sp., and *Bufo d'orbigny* (Galli-Mainini, C., 1948); *Bufo americanus* and *Bufo woodhousii* (McCallin, P. F., and Whitehead, R. W., 1949); *Bufo melanostictus, Bufo stomaticus,*

and *Rana tigerina* (Bhaduri, J. L., and Bardhan, N. R., 1949); *Bufo vulgaris* (Klopper, A., and Frank, H., 1949); *Bufo viridis* (Sulman, F. G., and Sulman, E., 1949); *Bufo bufo* (Haines, M., and Ferreira, H. P., 1949); *Rana vittigera* (Keegan, H. L., and Stonesifer, P. S., 1950); *Bufo boreas halophilus* (Johnson, D. A., 1950); *Rana clamitans* (Marsters, R. W., 1950); *Bufo regularis* (Rousselot, R., 1950); and certain common French amphibians (Creze, J., 1949).

Because of the ever increasing number of salientia being used as test animals in the diagnosis of pregnancy, the writer was interested in the possible use of frogs and toads native to Florida.

The species were selected as to their relative abundance and their general size. A few tests were made on *Hyla gratiosa* and positive results were obtained using both commercial chorionic gonadotropin and pregnancy urine. However, a smaller species of this type proves rather unpractical for the technique. This is the first attempt to use a species from the family Hylidae. Collections were made at night by blinding the frogs with a bright carbide light. As all species used (Table 1) possessed distinguishing secondary sexual characteristics, sex determination was relatively simple. The males of *Rana catesbeiana, Rana clamitans, Rana grylio,* and *Rana heckscheri* were identified by their tympanum, this being larger than the eye in the male of each of these species. The swollen and darkly pigmented thumb distinguished the male *Rana pipiens sphenocephala.* The male of *Bufo terrestris* was recognized by its darkly pigmented throat, whereas this sex in *Scaphiopus holbrookii holbrookii* was identified by the black horny excrescences on the thumb and first finger. Frogs and toads

TABLE I

Results of Tests Using First Trimester Pregnancy Urine

Salientia	Cubic Centimeters of Urine Injected	Length of Body of Test Animals in Millimeters	Positive	Negative
Bufo terrestris	3	50 – 65	20	1
Rana catesbeiana	5 – 10	101 – 130	17	3
Rana clamitans	5	64 – 73	13	0
Rana grylio	5	76 – 98	21	1
Rana heckscheri	5	76 – 91	8	0
Rana p. sphenocephala	5	56 – 73	16	0
*Rana p. pipiens**	5	60 – 72	20	0
Scaphiopus h. holbrookii	3	55 – 69	0	36

* This subspecies was obtained from northern biological supply houses.

were usually stored in small mesh-wire cages, where water was accessible. In some instances frogs were stored under refrigeration at approximately ten degrees centigrade. By this method frogs were successfully held from two to four weeks without feeding.

The technique is quite simple. Depending upon the size of the frog, three to ten cubic centimeters of first trimester pregnancy urine (Table 1) was injected subcutaneously into the dorsal lymph sac of the test animal. The amphibian was then placed in a large clean jar with a perforated lid. Two to five hours later this jar was examined for urine voided by the animal. If no urine was present, a small pipette was introduced into the cloacal orifice and cloacal fluid obtained by aspiration. Urine, taken either from the jar or directly from the frog, was then examined microscopically for the presence of spermatozoa. As seen under reduced light, the sperm appear as elongate cylindrical bodies, somewhat cigar shaped in form. Under the phase microscope, the long, slender, tails of the sperm are easily observed. The presence of sperm indicates a positive result, or pregnancy, while the absence of sperm shows a negative result. In the positive cases the sperm were heavily concentrated in the microscopic field giving a clear and definite end point. Exception to this was found in the *R. catesbeiana* where the sperm concentrations were rather weak. As few as two or three sperm were found in the high power field. There is some likelihood that seasonal variation and large size may effect the sensitivity of *Rana catesbeiana* and even *Rana grylio*. Continued studies are being made on these problems. Controls were made by using male and non-pregnant female urines. No sperm were ever found as a result from these injections. Also, each amphibian's urine was inspected for the presence of sperm previous to injection as a second manner of control.

Five native salientia proved reliable as test animals for early pregnancy diagnosis (Table 1). A total of 120 tests were performed on the first seven anurans, resulting in 115 positive tests and five negative tests. The single exception of *Bufo terrestris* was probably due to a low hormone concentration in the pregnancy urine used. Although *Rana catesbeiana* is questionable in its reliability, *Scaphiopus h. holbrookii* proved totally unreliable. Thirty-six tests made on the spadefoot toad yielded total negative

results as no sperm were ever found. Careful examination of this spadefoot's urine for sperm from one to eight hours after injection proved futile. Apparently seasonal variation does not effect this subspecies as separate groups collected and tested in May and October gave identical negative results. The latter group was collected while breeding. Animals from both groups were sacrificed, opened and their testes examined for sperm. Motile sperm were found in each specimen. In every case careful controls were made, identical urine being injected in the spadefoots and the control animals, *Rana p. sphenocephala*, *Rana grylio*. However, the toads consistently yielded no sperm while the controls proved positive. Although commercial chorionic gonadotropin brought about the abundant release of spermatozoa in the *Rana clamitans*, *Rana grylio*, and *Rana p. sphenocephala*, no similar effect was obtained with *Scaphiopus h. holbrookii*. Five of these toads injected with this hormone, in amounts of 100-250 rat units per toad, resulted negatively.

To date some twenty-three or more species and subspecies of male salientia, in addition to the five as reported in this paper, have been reported as responding to the gonadotropic hormone in pregnancy urine by the release of spermatozoa. These results would seem to indicate that this response is a generalized phenomena peculiar to male frogs and toads excepting *Scaphiopus h. holbrookii*, and thereby offers a practical means of early pregnancy determination. It is interesting to note that the spadefoot toad belongs to the Family Scaphiopodidae, a group of anurans heretofore untried for pregnancy diagnosis. Thus far, only species from the Families Pipidae, Bufonidae, Leptodactylidae, and Ranidae had been used as test animals for human pregnancy diagnosis. It should prove worthwhile to further investigate this family of spadefoots, the possibility being that others are also insensitive to the chorionic gonadotropic hormone.

Appreciation is given to two local physicians of DeLand for their cooperation in providing pregnancy urine used in this experimentation. Apologies are made to those authors whose work was unintentionally omitted from the history of this paper.

Literature Cited

ASCHHEIM, S., and ZONDEK, B.
1928. Schwangerschaftsdiagnose aus dem Harn (durch Hormonnachweis). *Klin Wchnschr.*, 7:8.

BHADURI, J. L., and BARDHAN, N. R.
1949. Male frogs and toads as test animals for early pregnancy and certain related conditions. *Science*, 109:517-518.

CREZE, J.
1949. A test for pregnancy using the common French male amphibians. *Rev.* France. Gynecol. et Obstet., 44(2):53-56.

FRIEDMAN, M. H., and LAPHAM, M. E.
1931. Simple, rapid procedure for laboratory diagnosis of early pregnancies. *Am. Jn. Obst. and Gynec.*, 21:405-410.

GALLI-MAININI, C.
1947. Pregnancy test using male toad. *Jn. Clin. Endocrin.*, 7(9):653-658.
1948. Pregnancy test using the male batrachian. *Jn. Amer. Med. Assn.*, 138(2):121-125.

HAINES, M., and FERREIRA, H. P.
1949. Pregnancy test using a European male toad. *Nature* (London), 164(4172):668.

JOHNSON, D. A.
1950. The use of the male California toad in the diagnosis of pregnancy. *Calif. Med.*, 72(6):422-424.

KEEGAN, H. L., and STONESIFER, P. S.
1950. Use of male Philippine frog, Rana vittigera, as test animal for diagnosis of early pregnancy. *Amer. Jn. Clin. Path.*, 20(3):292-293.

KLOPPER, A., and FRANK, H.
1949. The use of English male toad in pregnancy test. *Lancet,* 257:9-10.

MARSTERS, R. W.
1950. The male *Rana pipiens* frog pregnancy test. *Carolina Tips,* 13(7):26.

McCALLIN, P. F., and WHITEHEAD, R. W.
1949. A study of native species of male toads as test animals in the diagnosis of early pregnancy. *Science,* 109:518-519.

ROBBINS, S. L.
1948. Pregnancy tests. *New England Jn. Med.,* 239:333-339; 369-373.

ROBBINS, S. L., and PARKER, F., JR.
1948. The use of the male North American frog, *Rana pipiens,* in the diagnosis of pregnancy. *Endocrinology,* 42(4):237-243.

ROUSSELOT, R.
1950. Application of the Galli-Mainini reaction to *Bufo regularis* in the French Congo. *Ann. Endocrin.,* 11(1):91-96.

SHAPIRO, H. A., and ZWARENSTEIN, H. A.
1934. Test for pregnancy on Xenopus laevis. *Tr. Roy. Soc. So. Africa,* 22: (min. of proc., 75).

SULMAN, F. G., and SULMAN, E.
1949. Pregnancy test with the male toad *Bufo viridis. Jn. Obstet. and Gynec. Brit. Emp.,* 56(6):1014-1017.

WILTBERGER, P. B., and MILLER, D. F.
1948. The male frog, *Rana pipiens,* as a new test animal for early pregnancy. *Science,* 107(2773):198.